(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 October 2002 (03.10.2002)

PCT

(10) International Publication Number
WO 02/076970 A2

(51) International Patent Classification[7]: C07D 311/76

(21) International Application Number: PCT/US02/11264

(22) International Filing Date: 21 March 2002 (21.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/278,264 23 March 2001 (23.03.2001) US

(71) Applicant *(for all designated States except US)*: SONUS PHARMACEUTICALS, INC. [US/US]; 22026 20th Avenue, S.E., Bothell, WA 98021 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: LAMBERT, Karel, J. [US/US]; P.O. Box 971, Woodinville, WA 98071 (US). LAL, Manjari [IN/US]; 5001 - 148th Avenue N.E., Apt. D101, Bellevue, WA 98007 (US). NIENSTEDT, Andrew, M. [US/US]; 3038 N.W. 61st Street, Seattle, WA 98107 (US).

(74) Agent: RENZONI, George, E.; Christensen, O'Connor, Johnson & Kindness PLLC, Suite 2800, 1420 5th Avenue, Seattle, WA 98101 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

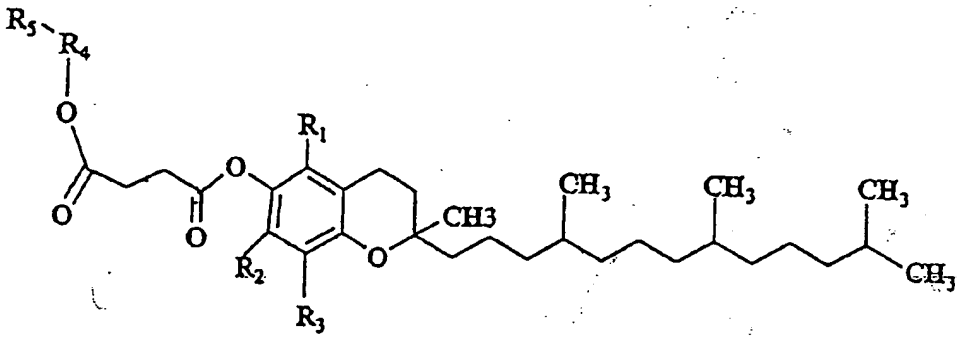
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

----- *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: TOCOPHEROL SUCCINATE DERIVATIVES AND COMPOSITIONS



(57) Abstract: Tocopherol succinic acid derivatives including tocopherol succinic acid esters and tocopherol succinic acid amides are described. Compositions that include the tocopherol succinic acid derivatives are also provided.

WO 02/076970 A2

TOCOPHEROL SUCCINATE DERIVATIVES AND COMPOSITIONS

FIELD OF THE INVENTION

The present invention relates to tocopherol succinate derivatives and compositions including the derivatives.

BACKGROUND OF THE INVENTION

Tocopherol succinic acid covalently linked to polyethylene glycol has been described as a surfactant. Known in the trade as "tocopherol polyethylene glycol succinate" and also called TPGS, this tocopherol succinic acid derivative was first disclosed in 1951. U.S. Patent No. 2,680,749 describes a water soluble d,l-α-tocopherol esterified with polyethylene glycol, optionally via a succinate, citraconate, or maleate diester linker. In subsequent patents issued to Eastman Kodak (U.S. Patent Nos. 3,102,078 and 5,234,695), TPGS was described in various compositions. Kovacs (U.S. Patent No. 5,583,105) describes the use of TPGS (tocopherol polyethylene glycol succinic acid) as a surfactant in solubilization of cyclosporin for oral administration. TPGS for administration of a therapeutic agent was claimed by Biogal (U.S. Patent No. 5,583,105) following disclosure in trade publications of the utility of TPGS as a bioavailability enhancer for drug delivery (Sokol et al., *The Lancet 338*:212-215, 1991).

TPGS is readily degraded by acidic or enzymatic de-esterification, and forms a clinically useful source of vitamin E (with 91% of the biological activity of vitamin E on a molar basis).

Efforts to couple molecules to vitamin E have included utilizing either the succinate or phosphate functional groups as bifunctional linkers (Meybeck A. et al., U.S. Patent No. 5,387,579; Mori A. et al., WO99/39716; Ogata K. et al., WO99/33818; Ogata K., U.S. Patent No. 4,742,163; Cunningham T., U.S. Patent No. 3,883,565; Shintaro A. et al., JP 62187470; Eugster C. et al., U.S. Patent No. 5,629,302; Wasner M. et al., "Nucleosides: Synthesis and characterization of monomeric cordycepin-Vitamin and cordycepin-lipid conjugates, model substances for biodegradable ester and carbonate linkages in conjugates and potential inhibitors of HIV-1 replication," *Helv Chim Acta 79*:609-619; Takata J. et al., 1995, "Prodrugs of Vitamin E. Preparation and enzymatic hydrolysis of aminoalkane-carboxylic acid esters of d-a-tocopherol," *J. Pharm Sci 84*:96-100). More recently, other dicarboxylic acids have been used as homobifunctional

linkers (U.S. Patent No. 6,045,826). These efforts, however, have not been directed at the synthesis of surfactants.

The goal of the above-noted references appears to be (1) to improve the water solubility of the oily vitamin so as to improve dietary or parenteral uptake of vitamin E in certain clinical and veterinary conditions, (2) to discover novel medicaments, and/or (3) to develop novel antioxidants. Only recently, the solubilization properties of tocopherols, tocopherol acetate, tocopherol succinate, TPGS, and tocotrienols have been recognized. However, the chemistry of tocopherol derivatization for this purpose has not advanced much since the introduction of TPGS in 1951. Moreover, the chemistry of tocopherol derivatization has not encompassed use of tocopherol succinic acid as an efficient starting material for synthesis of new biocompatible surfactants as described herein. The above-noted references have not recognized the use of these derivatives as surfactants, or as pharmaceutical excipients in emulsions, nanoemulsions, microemulsions, liposomes or micellar solutions. The above-noted references have further failed to recognize the utility of certain tocopherol succinic acid derivatives for therapeutic use.

The present invention seeks to fulfill these needs and provides further related advantages.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides tocopherol succinic acid derivatives. In one embodiment, the tocopherol succinic acid derivative is an ester. In another embodiment, the tocopherol succinic acid derivative is an amide.

The tocopherol succinic acid derivatives include tocopherol succinate acid covalently coupled to a hydrophilic moiety through a linker moiety. The tocopherol succinate acid is coupled to the linker moiety through at least one of an ester bond or an amide bond. The hydrophilic moiety associates with water to form a plurality of hydrogen bonds. In one embodiment, the linker includes an amino acid, such as aspartic acid. In one embodiment, the linker includes a diamine, such as ethylenediamine. In one embodiment, the hydrophilic moiety comprises an amino acid, such as aspartic acid or glutamic acid.

In another aspect of the invention, compositions that include the tocopherol succinic acid derivatives are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 is the chemical structure of a representative tocopherol succinic acid ester of the invention;

FIGURE 2 is the chemical structure of a representative tocopherol succinic acid amide of the invention;

FIGURE 3 is the chemical structure of a representative tocopherol succinic acid amide aspartate of the invention;

FIGURE 4 is the chemical structure of a tetraglutamic acid useful in preparing representative tocopherol succinic acid derivatives of the invention;

FIGURE 5 is the chemical structure of a benzyloxy tetraglutamate useful in preparing representative tocopherol succinic acid derivatives of the invention;

FIGURE 6 is the chemical structure of a representative tocopherol succinic acid amide tetraglutamate of the invention; and

FIGURE 7 is the chemical structure of a representative tocopherol succinic acid amide triaspartate of the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In one aspect, the present invention provides tocopherol succinic acid derivatives. In one embodiment, the tocopherol succinic acid derivative is an ester. In another embodiment, the tocopherol succinic acid derivative is an amide. In another aspect of the invention, compositions that include the tocopherol succinic acid derivatives are provided.

The tocopherol succinic acid (TOSA) derivatives of the invention can be used as surfactants and as pharmaceutical excipients in the compositions described herein. The chemical structures of representative tocopherol succinic acid derivatives are illustrated in FIGURES 1-3 and 6, and 7. FIGURE 1 is the chemical structure of a representative tocopherol succinic acid ester of the invention. FIGURE 2 is the chemical structure of a representative tocopherol succinic acid amide of the invention. FIGURE 3 is the chemical structure of a representative tocopherol succinic acid amide aspartate of the invention. FIGURE 6 is the chemical structure of a representative tocopherol succinic

acid amide tetraglutamate of the invention. FIGURE 7 is the chemical structure of a representative tocopherol succinic acid amide triaspartate of the invention.

In these figures, the noted substituents are as described below.

R_1, R_2 and R_3 are substituents selected independently from hydrogen, methyl, or ethyl.

R_4 is a linker, used optionally, and is selected from homobifunctional or heterobifunctional linkers, further comprising C_1 - C_{30} alcohols, thiols, amines, amides and acids, or organophosphatyl. In certain embodiments, R_4 is selected from substituted alcohols, thiols, amines, amides and acids, branched or unbranched, aliphatic or aromatic, alkyl or alkene, containing up to 10 substituents selected from organophosphatyl, amine, azo, diazo, hydroxyl, sulfhydryl, disulfide, oxo or carboxyl. In certain embodiments, R_4 is a linker selected from ascorbyl, phosphoryl, ribosyl, glucosyl, glycinyl, glutamyl, glutaminyl, aspartyl, cysteinyl, lysinyl, argininyl, hexane diamine, pentane diamine, butane diamine, propane diamine, ethane diamine (EDA), maleimide, or the reaction products of BNTH (N-[β-maleimidoproprionic acid] hydrazide TFA), EMCH (n-ε-maleimidocaproic acid) hydrazide, KMUH (n-[κ-maleimidoundecanoic acid]hydrazine), and TFCS (N-[ε-trifluoroacetylcaprolyloxy] succinimide ester), or composites thereof.

R_5 is a substituent that associates with water to form at least 2 hydrogen bonds, in one embodiment 3 hydrogen bonds, and in other embodiments from 2 to about 200 hydrogen bonds. R_5 can optionally form a salt in buffered water or saline, and may be selected from a carboxylate (such as sorbate, tartarate, succinate, citrate, gluconate, glucoheptonate, glycerate, itaconate, aconitate, galacturonate, galactarate, glutarate, creatine, fumarate and its polymers, ascorbate, lactate and its polymers, pangamate, pantothenate, or para-aminobenzoate), an amine (such as glucamine, glucosamine, thiamine or choline), a phosphate (such as glycerophosphate, fructose-6-phosphate or phosphocreatine), an amino acid, a purine (such as adenine and guanine), a nucleoside or nucleotide (such as adenosine, deoxyadenosine, guanidine, cytosine, thymidine, uridine, or polyadenosine), a polypeptide (such as vasopressin, polypep, ferrichromes, oxytocin, or rifampin), an alcohol (such as erythritol, adonitol, ribiflavine, flavine-adenine-dinucleotide, glucovanillin, taurocholate, glycocholate, tauroursocholic acid, glyco-nor-ursodeoxycholic acid, thiol, glycerol, mannitol or cyanocobalamin), a sugar (such as glucosamine, n-acetylglucosamine, n-acetylneuraminate, lactose, ribose, arabinose,

rhamnose, raffinose, maltose, lactobionose, heparin sulfate, trehalose, gluconate, galactose, sucrose or glucose), a polyhydric alcohol (for example $-(OCH_2OH)_nOH$, $-(OCH_2CHOH)_nOH$, $-(OCH_2CHOHCHOH)_nOH$, $-(OCH_2CH_2CHOH)_nOH$, $-(OCH_2CHOH)_nOH$, $-(OCH_2CH_2CHOH)_nOH$, or $-(OCH_2CHOHCH_2)_nOH$, where n is 1 to 100, and branched or block co-polymers of the same). In certain embodiments, R_5 includes residues of carnitine, sarcosine, taurine, methionine, glutathione, β-alanine, glycine, glutamate, glutamine, aspartate, asparagine, ornithine, arginine, γ-aminobutyrate (GABA), serotonin, adrenaline, histamine, melatonin, tryptamine, alanylglutamine, glycylglutamine, glycylsarcosine, valyl-lysine, aspartylalanine, glutamyltryptophane, lysyl-sarcosine, glycylproline, triglycine, polyglutamate $(Glu)_n$, polyglutamine $(Gln)_n$, polyglycine $(Gly)_n$, polyalanine $(Ala)_n$, polyproline $(Pro)_n$, poly-$(GlyProAla)_n$, polyserine $(Ser)_n$, and other biogenic amines as defined below, polyesters, copolymers of succinate, glycerol, and polyethylene glycol, polyhydroxyalkonates, polyhydroxyproprionate, poly-(3-hydroxyvalerate), poly-(3-hydroxyhexanoate), poly-(4-hydroxyvalerate), poly-(5-hydroxyvalerate), generally R-3-hydroxyacid polymers and their derivatives, polyglycolides (PGA), polylactides (PLA), substituted polyhydroxybutyrates, folate, glycogen, chitosan, dextran, dextrin, gluconate, polyvinylpyrrolidinone, poloxamer, polyvinylalcohol, polyethylene glycol, l-amino-polyethyleneglycol, or composites (co-polymers) of the above.

When a linker is not used, R_5 maybe joined directly to the terminal carboxyl of tocopherol succinic acid.

In an alternate embodiment, R_5 provides a derivative that is not bonded or is weakly bonded by a C8 column packing, and most preferentially is not bonded or is weakly bonded by a C18 column packing (for example BondElute™). Bonding can be assessed by measuring retention times on an HPLC set up with a reverse phase column and a gradient solvent system progressing from relatively nonpolar to more polar. Those compounds that are poorly retained (i.e., have low retention times) are the preferred compounds for R_5. Molecular weights for R_5 are typically between 20 Da to 5 kDa. In one embodiment, R_5 molecular weights are from about 80 Da to 5000 Da. In another embodiment, R_5 molecular weights are from about 180 Da to 2500 Da.

T_1 and T_2 are substituents of the benzofuran ring. T_1 can be a C_1 to C_{80} hydrocarbyl, hydoxyhydrocarbyl, oxyhydrocarbyl, carboxyhydrocarbyl or phosphohydroxy-hydrocarbyl, saturated or unsaturated, aliphatic or aromatic, branched or

unbranched; in certain embodiments, an isoprenoid, terpene, diglyceride or phospholipid; and in other embodiments, a phytyl (4,8,12-trimethyl-tridecyl), or trienyl (4,8,12-trimethyl-3,7,11-tridecatrienyl). T_2 can be a hydrogen, halo, hydrocarbyl, or carbonyl, and, in certain embodiments, a methyl, ethyl, or carboxyl, as the l-stereoisomer.

The stereochemistry of T_1 and T_2 may be as d- or l-stereoisomers or as racemates, and the invention is not limited by the stereochemistry of any chiral centers.

These tocopherol succinic acid derivatives of the invention can be cationic, anionic, zwitterionic, multipolar or nonionic: Ionically charged derivatives may be formed and used as salts, for example as the sodium, hydrochloride, citrate, lactobionate, propionate, succinate, potassium, lithium or palmitate salt.

The invention further relates to compositions that include the tocopherol succinic acid derivatives. Suitable compositions include pharmaceutical, nutriceutical, cosmeceutical, vitamin, foodstuff, antigen, catalyst, cell, nanoparticle, oligonucleotide, gene, extract, cosmetic or fiber is solubilized, protected or dispersed in a solution, particle, emulsion, microemulsion, nanoemulsion, liposome, niosome, molecular matrix or coating including one or more of the derivatives, optionally with other oils, co-solvents, surfactants, and cosurfactants. In one embodiment, the composition is a biocompatible or therapeutic formulation including one or more of tocopherol succinic acid derivatives for application to a human or to an animal by any of a variety of routes. Suitable administration routes include oral, topical, and parenteral administration. Such compositions include solutions, suspensions, emulsions, emulsion preconcentrates, liquigels, lotions, astringents, soaps, ointments, toothpastes, topicals, capsules, tablets, sustained release granules, powders, nosedrops, eyedrops, excipients, sunscreens, surgical dressings, intravenous infusions, depot or sustained release injections, and coatings for prosthetic devices.

As used herein, the term "tocopherol succinic acid derivative" refer to select classes of tocopherol derivatives formed by amidation or esterification (inclusive of alcohols to form carboxyesters, thiols to form thioesters, and phosphates to form organophospho diesters) at the terminal carboxyl of the succinate moiety. The derivatives are formed by chemistry that is commercially attractive. The derivatives also have unexpected utility as biocompatible surfactants, as pharmaceutical excipients, and as bioavailability enhancers. In contrast, typical commercial surfactants, such as sodium oleate (a principal component of ordinary soaps), betaine, or SDS (sodium dodecyl

sulfate), dissolve biological membranes and are corrosive to cells. Thus, the utility of vitamin E-based surfactants that, almost paradoxically, serve to stabilize biocellular membranes is anticipated to be great.

As described below, certain derivatives displayed surfactant properties of foaming and emulsion formation even before the protective groups on the "hydrophilic head" had been removed.

The biocompatibility of tocopherol oils in contact with cell membranes and organelles is truly remarkable, and it is well known that tocopherols characteristically stabilize biological membranes in the presence of other surfactants. The present invention provides biological tocopherol-based surfactants that can be anionic, cationic, zwitterionic, multipolar, or non-ionic.

As noted above, the tocopherol succinic acid derivatives of the invention include tocopherol succinic acid esters and tocopherol succinic acid amides. Certain of the amide derivatives are shown herein to have surprising utility as biological response mediators, i.e., to have therapeutic uses.

To assist in understanding the invention, the following definitions are provided.

Vitamin E: Vitamin E as used herein is the common name for RRR-α-tocopherol (d-α-tocopherol, *sensu stricto* 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl-6-benzopyranol), the vitamin named by Dr. George Calhoun and Dr. H.M. Evans in 1936. The suffix "-ol" denotes the presence of the 6-hydroxyl on the benzopyran ring. Vitamin E is a member of the family "tocopherols." Vitamin E has a bioequivalence of 1.00 αTE units in biological assays for Vitamin E activity.

Vitamin E Equivalency (αTE): Modem practice uses d-α-tocopherol (RRR-α-tocopherol) as the standard for biopotency of Vitamin E, i.e., as a synonym. The potency of purified natural RRR-α-tocopherol is set at 1.00 αTE units. For a more complete explanation of Vitamin E biopotency, see Papas A. 1999. *The Vitamin E Factor* Harper Perennial NY. Note, however, that tables of biopotency often fail to reflect the property of some tocol esters to release high-potency tocols upon hydrolysis or metabolic cleavage. Therefore, for example, TPGS when given parenterally has the capacity to deliver substantial amounts of Vitamin E. Similarly, tocopherol nicotinate and tocopherol lineolate, which are often cited as having essentially no Vitamin E activity (i.e., αTE≈0), in fact are highly potent sources of Vitamin E, and are only cited in the literature as possessing no Vitamin E activity because government regulations in the

United States do not permit them to be labeled as such. It should also be recognized that although recent revisions in nutritional guidelines focus on weight or mass, the stoichiometric bioequivalence of any tocan compared to Vitamin E (native RRR-α-tocopherol) on a molar basis is the intended teaching of this invention.

Tocol: "Tocol" is used herein in a broad sense to indicate the family of tocopherols and tocotrienols and derivatives thereof, including those common derivatives esterified at the 6-hydroxyl on the chroman ring. This use of the term "tocols" (or tocol, singular generic) is appropriate since all tocopherols and tocotrienols are fundamentally derivatives of the simplest tocopherol, 6-hydroxy-2-methyl-2-phytylchroman (sometimes referred to as "tocol").

Tocan: "Tocan" or "tocans" are used herein in broad sense to indicate the various members of the families of tocopherols and tocotrienols, their rarer natural and synthetic analogs, and in addition all benzopyran derivatives substituted at the 2-position by T_1 and T_2, where T_1 can be a C_1 to C_{80} hydrocarbyl, hydoxyhydrocarbyl, oxyhydrocarbyl, carboxyhydrocarbyl or phosphohydroxy-hydrocarbyl, saturated or unsaturated, aliphatic or aromatic, branched or unbranched; in certain embodiments, an isoprenoid, terpene, diglyceride or phospholipid; and in other embodiments, a phytyl (4,8,12-trimethyl-tridecyl), or trienyl (4,8,12-trimethyl-3,7,11-tridecatrienyl). T_2 can be a hydrogen, halo, hydrocarbyl, or carbonyl, and, in certain embodiments, a methyl, ethyl, or carboxyl, as the l-stereoisomer.

Tocans also include tocols esterified at the 6-hydroxyl on the benzopyran ring. When administered *in vivo*, these derivatives are readily de-esterified at low pH or by esterases in the thoracic duct and in the blood, releasing the free tocol. Common tocol esters known in the art include the acetate, succinate, maleate, phosphate, linoleate, nicotinate, ascorbate, retinoate, quinone, and a pegylated diester derivative known as TPGS (tocopherol polyethylene glycol succinate).

Xenotocans: A subclass of tocans characterized as having a Vitamin E equivalency (αTE) of less than 0.75 αTE units, preferentially less than 0.6 αTE units, even more preferentially less than 0.2 αTE units (on a molar basis, for the purposes of this invention) are termed herein "xenotocans". Xenotocans are a preferred form of the invention where intravenous dosing is anticipated. The amounts of Vitamin E released from tocopherol succinic acid may exceed toxic limits on chronic intravenous dosing, and this invention solves that problem.

Linker: In chemical synthesis, conjugations of two molecules A and B may take place using a linker "C" to modify the reactivity of a functional group so that the joining takes the form A+C+B in the final structure. Note the position of C between A and B. Linkers may be homo- or hetero-bifunctional, or multi-functional as in the synthesis of dendrimeric molecules.

Spacer: A spacer is a special class of linkers in which the separation of A and B is increased by the length of the spacer C.

Cap: An end group on a side chain, particularly on a polymer.

Hydrocarbyl: By "hydrocarbyl" is meant moieties containing carbon and hydrogen atoms only, with the indicated number of carbons atoms. Hydrocarbyl groups may be straight-chain or branched-chain. Preferably the hydrocarbyl groups are saturated (e.g., alkyl groups). However, unsaturated, groups such as 1- and 2-butene and propargyl, including multiply unsaturated groups such as butadienyl or phenyl, are included in this term.

Surfactant: Surfactants are bipolar molecules characterized by one simple property: they are driven to occupy interfaces between two phases, typically either liquid/gas phase interfaces, liquid/solid interfaces, or liquid/liquid phase interfaces (for immiscible liquids), so that the free energy of the boundary surface between phases is reduced. Obviously, the "surfactanticity" of any molecule is not independent of the properties of the immiscible phases under study. With respect, for example, to water and oil, a surfactant will contain both a water-loving "head" and an oil-loving "tail". Some surfactants, however, instead contain fluorophilic "tails." The basic principle is that the molecule is amphipolar with respect to the two phases with which it associates. Surfactants may be classed into five sub-groups: anionic, cationic, zwitterionic, multipolar and non-ionic on the basis of chemical structure and the presence or absence of electrostatically charged substituents. Surfactants are also sometimes termed "emulsifiers." Catanionic, gemini, and bolaform surfactants are special cases.

Co-surfactant: A second surfactant that aids in reduction of the surface free energy between two phases, most typically that aids in fine emulsification of an oil and water or dirt and water system.

Hydrophile-Lipophile Balance (HLB): An empirical formula used to index the relative detergency of surfactants. Its value varies from 1 to about 45 or more and in the

case of non-ionic surfactants from about 1-20. In general, for lipophilic surfactants the HLB is less than 10 and for hydrophilic ones the HLB is greater than 10.

Critical Micellar Concentration (CMC): An experimentally determined concentration of amphiphile, surfactant or detergent molecules in solution distinguished by the appearance of organized "micelles" (defined below).

Oil: Any of a class of hydrocarbon derivatives that are hydrophobic and immiscible or poorly miscible with water. Oils may be synthetic or derived from plants, animals, or microorganisms. Such oils include "grease," waxes, "dirt," triglycerides, diglycerides, derivatives of mono- and diglycerides, essential oils, vitamin oils, nutrient oils, squalene, squalane, waxes, terpenes, ethers and crown ethers, and may be either synthetic or natural. In general, the melting points of oils are less than 100°C and most are in fact liquid at body temperature.

Common oils include extracted and distilled oils from nuts and seeds, for example safflower, perrila, millet, niger, Ucuuba, sesame, cimbopogon, mustard, canola, corn, caraway, soybean, sunflower, garlic, peanut, pumpkin seed, olive, almond, macadamia, palm, walnut, pistachio, coconut, evening primrose seed, black currant seed, rosemary, borage seed or flax seed oils, and from fish and phytoplankton, for example, shark, cod, mackerel, sardine, salmon, scrod, or halibut oils, or from oleagenous microorganisms directly. Also included are tocols (comprising the whole family of tocopherols and tocotrienols, including the acetate esters), certain terpenoids comprising Vitamin A (also called retinol), retinoids, menaquinones such as Coenzyme Q, carotenoids such as carotenes, lycopene, and the related xanthophylls, such as lutein, lutein esters, astaxanthin, canthaxanthin and zeaxanthin, Vitamin D, Vitamin K, vitamers of these vitamin oils and their precursors, and glycerides high in PUFAs such as triglycerides containing esterified docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Also contemplated as oils are essential oils. These are often complex mixtures useful in enhancing bioavailability, and include extracts of (as in US 5,716,928) allspice berry, fennel, amber essence, anise seed, arnica, balsam of Peru, basil, bay leaf, parsley, peanut, benzoin gum, bergamot, rosewood, rosemary, rosehip, cajeput, marigold, turmeric, camphor, caraway, cardamom, carrot, cedarwood, celery, chamomile, cinnamon, citronella, palm kernels, avocado, macadamia, sage, clove, coriander, cumin, cypress, eucalyptus, aloe, fennel, fir, frankincense, garlic, geranium, rose, ginger, lime, grapefruit, orange, hyssop, jasmine, jojoba, juniper, lavender, lemon, lemongrass, marjoram,

mugwort, watercress, mullen, myrrh, bigarde neroli, nutmeg, bitter orange, oregano, patchouly, pennyroyal, primrose, retinols, papaya, red pepper, black pepper, baccharis (Vassoura Oil), peppermint, poppyseed, petitegrain, pine, spruce, poke root, rosemary, sandalwood, sassafras, spearmint, spikenard, hemlock, tangerine, tea tree, thyme, vanilla, banana, coconut, vetivert, wintergreen, witch hazel, ylang ylang extract, or synthetic analogs.

Lipophilic: Literally, fat-loving, referring to the property of certain molecules characterized as soluble in triglyceride oils, hydrocarbons, or waxes:

Tocol-soluble: Refers to the property of certain molecules characterized as being soluble directly, or with the aid of a co-solvent, in a tocol. As an operative definition, the most useful way to determine tocol solubility is to dissolve the compound of interest in a tocol or to use a co-solvent such as ethanol.

U.S. Patent Application 09/671,753, filed September 27, 2000, and PCT application PCT/US00/26467 (both of which are hereby incorporated herein by reference) disclose formation of tocol-soluble ion pairs between charged tocol derivatives (including ester derivatives of tocopherols) and oppositely charged therapeutic compounds. By "ion pair" is meant a neutral pair formed between two oppositely charged compounds. Tocopherol succinate acid derivatives of the present invention are among the tocol derivatives that are capable of forming tocol-soluble ion pairs with such oppositely charged therapeutics, thereby rendering them soluble in tocols or enhancing existing tocol solubility. The resulting compositions can be incorporated into various types of pharmaceutical compositions, including multiphasic compositions or their precursors, such as emulsions, liquid crystalline gels, self-emulsifying drug delivery systems, or liposomal or niosomal dispersions, for oral or other (including parenteral) administration.

Colloidal System: As used herein, this term refers to a system containing two or more immiscible phases, at least one of which takes the form of a particle or droplet and is termed a "dispersed" phase, and one phase is a liquid or a solution and is termed a "continuous" phase. As used herein, "colloidal systems" are limited to those wherein the particles are larger than simple molecular or micellar solutes but are small enough that they remain suspended in a fluid medium without settling to the bottom. The vapor pressure of a liquid or solution containing a colloid is typically not influenced by the colloidal particles or droplets in suspension. Nor are other colligative properties affected, for example, osmolarity. A review of the prior art of colloidal systems is provided in

Zografi G. et al. 1990. Disperse Systems, in Remington's Pharmaceutical Sciences, (Gennaro A.R. and T. Medwick, eds) Philadelphia, PA. Emulsions, nanoemulsions, miniemulsions, and liposomes are examples of colloidal suspensions. Oil-in-water (o/w), water-in-oil (w/o), solid-in-oil, solid-in-water, and oil-in-solid colloidal systems are known in the art. Colloidal systems are most preferably stabilized with surfactants. Aspects of the invention also include precursors of colloidal systems, for example, SEDDS (self-emulsifying drug delivery systems), wherein the oily phase containing a therapeutic agent is administered as a preconcentrate, typically with surfactant(s), sometimes with solvents, but absent water.

Multiphase System: As used herein, this term refers to a system where one or more phase is (are) dispersed throughout another phase, which is usually referred to as the continuous phase, or a precursor thereof. Complex emulsions, microemulsions, and other multiphasic nanoparticulates, including liposomes, niosomes, and crystalline suspensions in oil-in-water emulsions, are examples of multiphasic systems. These systems may be lyophilic or lyophobic. Biphasic systems are a subcategory of multiphasic systems.

Emulsion: A colloidal dispersion of two immiscible or poorly miscible liquid phases, such as oil and water, in the form of droplets. The internal phase is also termed the dispersed phase and the external phase is termed the continuous phase. The mean diameter of the dispersed phase, in general, is between about 0.2 and about 50.0 microns (μm), as is commonly measured by particle sizing methods, and the particles range broadly in size. Emulsions in which the dispersed phase and continuous phase have different refractive indexes are typically optically opaque. Emulsions in which the refractive indexes of the two phases are similar may be clear or translucent, and hence optical appearance is not a defining characteristic. Emulsions possess a finite or limited stability over time, and can be stabilized for days or weeks, sometimes for months, by the incorporation of surfactants and by viscosity modifiers.

Microemulsion: A thermodynamically stable, isotropically clear mixture of two immiscible liquids, stabilized by a relatively high concentration of surfactant molecules. Microemulsions have an apparent mean droplet diameter of less than about 200 nm, in general from about 10 to about 100 nm, and are typically self-assembling, or may be assembled using heat and/or solvents. Typically, microemulsions are more easily established with a co-surfactant. Microemulsions exist only within defined ratios of

water, amphiphile and oil, as may be determined with a phase diagram for the system. Therefore, o/w microemulsions are inherently unstable when diluted with water. A class of microemulsions known as "swollen micelles" constitutes a system of special interest for drug delivery. Microemulsions of nonvolatile oils decrease the vapor pressure of the continuous phase, and conversely, microemulsions of volatile oils may increase the vapor pressure of the continuous phase, a change that may involve substantial departures from ideal solute behavior, where ideality is taken as an approximately linear relationship between solute concentration and vapor pressure.

Tocan Microemulsion: A thermodynamically stable, isotropically clear mixture of two immiscible liquids, one of which contains a tocan or a tocan derivative, stabilized by a relatively high concentration of surfactant molecules, optionally comprising one or more tocan surfactants. Tocan microemulsions have an apparent mean droplet diameter of less than about 200 nm, in general from about 10 to about 120 nm, and may require mechanical shear for manufacture. Once manufactured, however, they are filter sterilizable and are highly stable. Typically, tocan microemulsions are more easily established with a co-surfactant. A class of tocan microemulsions known as "swollen micelles" constitutes a system of special interest for drug delivery.

Nanoemulsion: Nanoemulsions, sometimes termed miniemulsions, are colloidal systems distinct from microemulsions and emulsions. As used herein, this term refers to those systems having a mean particle size that is less than 200 nm (as Gaussian volumetric mean), preferably less than 120 nm, and most preferably from about 10 to 100 nm, and not displaying apparent growth in particle size as measured by a lack of increase in size of greater than 15% in Gaussian volumetric mean by photon correlation spectroscopy when incubated at 25°C under controlled conditions for at least 30 days, preferably for 6 months, and most preferentially for up to 2 years. Some of these vehicles are isotropically clear and display high levels of drug loading (a relative property that must be determined independently for each drug). Some are translucent or hazy. Some nanoemulsions are self-assembling, but others may require heat, solvent, and/or increased shear to assemble due to the high viscosity of certain nutrient oils. Operationally, nanoemulsions, however formed, share the common property of being terminally, filter sterilizable, typically by passage through a compatible filter membrane of a pore size not to exceed 0.2 microns. And unlike. o/w microemulsions, o/w nanoemulsions are robust when diluted with aqueous IV solutions, an important property when used for drug

(CMC) in water or buffer. The CMC is an individual characteristic of each surfactant. These molecular aggregates typically have a nominal diameter of 2 to 6 nm, and perhaps 15 or 20 nm in some systems. Micellar solutions of surfactants cause departures from non-ideality of the vapor pressure of the continuous phase and have other properties not characteristic of colloids.

Self-Emulsifying Drug Delivery System (SEDDS): With reference to a phase diagram, certain mixtures of oil(s) and non-ionic surfactant(s) will form clear and isotropic solutions that then spontaneously emulsify when mixed with water. These mixtures, when comprising drug as well as oil and surfactant, are known as self-emulsifying drug delivery systems (SEDDS), or emulsion pre-concentrates. Optionally, they may also contain solvents and other excipients. SEDDS and the related SMEDDS (self-microemulsifying drug delivery systems) have successfully been used to improve lipophilic drug dissolution and oral absorption.

Tocan Self-Emulsifying Drug Delivery Systems (TSEDDS): With reference to a phase diagram, certain mixtures of oil(s) and tocan surfactant(s) will form clear and isotropic solutions that then spontaneously emulsify when mixed with water. These mixtures, when comprising drug as well as oil and surfactant, are known as tocan self-emulsifying drug delivery systems (TSEDDS) or tocan emulsion pre-concentrates. Optionally, they may also contain solvents and other excipients. TSEDDS and the related TSMEDDS (tocan self-microemulsifying drug delivery systems) are useful to improve lipophilic drug dissolution and oral absorption.

Biocompatible: Capable of performing functions within or upon a living organism in a manner that does not terminate or excessively disable the life of the organism, i.e., without undue toxicity or harmful physiological or pharmacological effects.

Prodrug: A prodrug is a chemical derivative of a therapeutic agent which, following administration, is cleaved or metabolized to release the therapeutic agent in situ.

Biogenic Amine: Biogenic amines are those cell signaling or transmitter molecules produced by the body and which contain an amine. These may be biological response modifiers or neurotransmitters. The class of amines includes tryptamine, tryptophane, histamine, histidine, serotonin, metatonin, epinephrine, noradrenaline, mescaline, and γ-aminobutyric acid (GABA). In some cases the precursor is also

efficacious, for example the amino acid tryptophane which is metabolized into serotonin, an active cell signaling and neurotransmitter molecule. "Cytomedines" as a subclass of biogenic amines. Cytomedines are a class of ligands that interact with cellular receptors to evoke a biological response. Cytomedines include EW, W, H, WH, HW, histamine-W, serotonin-W, W-histamine, W-serotonin, H-serotonin, H-histamine, histamine-serotonin, histamine, and serotonin are of particular interest here, the heterodimers because they possess the capacity to block both the H1 and H2 receptors involved in allergic and immunological responses, such as are involved in host-mediated immunosurveillance and natural immunotherapy for cancer, infectious diseases and reversal of the progressive loss of these natural functions through old age, stress or secondary condition.

The present invention provides tocopherol succinic acid derivatives, some which include biomolecules. In certain aspects, the invention provides "stealth coat" surfactants that include a hydrophilic "head" and a phytyl "tail". The "greasy" phytyl or phytotrienyl tail can be used to position correspondingly large hydrophilic heads at a lipid/water interface. Whereas stearylamine has a molecular weight of 270 daltons, the analogous tocan is about 450 daltons, a 65% increase. Whereas betaine, a good example of a corrosive and toxic detergent, has a CMC of about 0.0006 M, the CMC of TPGS is 0.0001 M, making TPGS, for example, a better detergent by a factor of six at low concentrations while gentle on cells. The large hydrophilic heads could in addition be used to prevent the interaction of the lipid droplets with phagocytic cells by "steric hindrance" and by binding of a layer of water to the particle through hydrogen bonds. Tocopherol succinic acid polyglutamate is a representative example of such a derivative. Surfactants of this type are hydrophilic with high HLB values and are effective by steric hindrances to coalescence and by electrostatic repulsion in stabilizing emulsions during storage. These surfactants have excellent detergency but are gentle on biological membranes.

Bacillus anthracis, a highly virulent bacterium which multiplies unchecked in the blood stream of mammals, including man, uses a capsule of polyglutamate (gamma-linked) to avoid triggering an immune response and to avoid phagocytosis. An artificial particle encapsulated in a polyglutamate coat could also evade the immune system, leading to prolonged circulation in the bloodstream, a highly desirable outcome. In the present invention, polyglutamate is attached to tocopherol succinic acid using mixed anhydride chemistry.

Tocopherol succinic acid amide conjugates retain the membrane insertion and stabilizing properties of the parent molecule and are biodegradable, albeit more slowly than esters or phosphate diesters. We discovered that the amide bond at the succinyl γ-carboxyl, slows cleavage of the succinyl α-carboxyl (i.e., slows the release of free Vitamin E or tocol), permitting the use of these molecules where more long lasting surfactants than the TPGS diester are desired. For these applications, they are improvements upon TPGS. This invention has the added advantage that when parenteral administration is contemplated, the amount of vitamin that is released from the inventive tocopherol succinic acid derivatives, which are preferably xenotocans for this use, is less than that released from an equivalent amount of TPGS, an important consideration when the total dose of tocols administered, particularly those comprising the Vitamin E moiety, exceeds safe limits.

The present invention also provides tocopherol succinic acid derivatives of biological amines, such as serotonin and histamine, that have been shown herein to have surprising utility as biological response mediators, i.e., to have therapeutic uses and a novel methotrexate conjugate. Tocopherol succinic acid derivatives of biogenic amines and their compositions in drug delivery vehicles are also provided.

One advantage of tocopherol succinic acid as a starting material for formation of tocan derivatives is the chemically reactive character of the carboxyl, particularly, for nucleophilic substitutions.

The chemistry of carboxy derivatives can involve "amide" or "peptide" bonds. The amide bonds are preferentially formed by mixed anhydride chemistry, or may be formed with carbodiimides or phosgene as a condensing agent. Amide bonds are biodegradable, but are more slowly degraded than ester; sulfhydryl or diphosphoester bonds, and for that reason have surprising advantages as surfactants.

By attaching bifunctional or multifunctional linkers, for example glycine, glutamate, maleimide, aspartic acid, asparagyl, cysteine, lysine, or arginine to the carboxyl, additional functional reactivity is obtained. Both homomultifunctional and heteromultifunctional linkers are provided.

During conjugation, delicate substituents or drugs must be protected from chemical degradation with "protective groups," which are subsequently cleaved off under mild conditions. For this reason, strong reaction conditions are impractical, a factor that rules out many common synthetic pathways. Mild or highly specific chemical synthetic

routes that are practical often lack the yields required to be commercially desirable. Because of these limitations, the use of tocopherol as starting material for preparation of drug conjugates has been limited and highly specialized. No facile approach to tocopherol-drug conjugation has emerged despite 50 years of research. Given these requirements, the mixed anhydride chemistry of amide bonds for conjugation has clear advantages in terms of specificity, cost, and yield.

Tocans as surfactants, for example as soaps, detergents and cosmetics,. will have good environmental compatibility, be biodegradable, be gentle on the skin or mucosa, optionally active in hard water, optionally will absorb UV radiation, optionally will adhere to skin, hair or fiber, and will be readily manufacturable. These are requirements that are addressed by the current invention.

The solvent and surfactant properties of the tocopherol succinic acid derivatives for cosmetics and medicament delivery vehicles has not previously been evaluated. Tocans can serve as biological detergents or as depot storage forms for the sustained-release delivery of drugs. Other multiphase systems include liquid crystalline structures such as liposomes, which are also suitable for drug delivery. In contrast, the relatively nonpolar tocopherols have proved rather poor in forming liposomes, and only 20 mol% tocopherol could be incorporated into liposomes made from phospholipids (Fukuzawa K. et al., "Location and Dynamics of α-Tocopherol in Model Phospholipid Membranes With Different Charges," *Chem Phys Lipids 63*:69-75, 1992), with little surface association of the phenolic hydroxyl. Tocopherol succinic acid derivatives of the invention are particularly useful in forming anionic surfactants, anionic liposomes, ionic nanoparticles, or ionic nanocrystals.

As noted above, the tocopherol succinic acid derivatives of the invention include R_5, which is optionally water soluble. If a surfactant is desired, a hydrophilic "head group" is selected for conjugation so that the desired HLB value or CMC is obtained. The relative hydrophilicity of the head group can be assessed by comparative bonding affinities to C8 and C18 resins (for example BondElute) or by measuring retention times on reverse phase columns by HPLC. The preferential form of conjugation is via an amide bond. The R_5 substituent(s) is or are preferentially amino acids, peptides or polyhydric alcohols. Where alcohols are used, the conjugation may take the form of an ester bond. R_5 is optionally lipophilic.

Other preferred classes of R_5 substituents include hydrophilic molecules. The R_5 substituent or substituents may be a sugar, amino acid, polyamino acid such as polyglutamate or polyglutamane, or poly-GluGln, or a macromolecule comprising multiple molecular units, for example, a peptide, carbohydrate, nucleic acid, or polymer such as polyvinylpyrrolidone, polyethylene glycol, poloxamer, chitosan, poly-γ-hydroxybutyrate, alginate or dextran.

The invention also provides derivatives in which tocopherol succinic acid is conjugated to a biogenic amine. These molecules have demonstrated biological activity consistent with their usefulness in treatment of cancer and in treatment of sleep and mood disorders.

In another aspect of the invention, compositions are provided that include the tocopherol succinic acid derivatives described herein. These compositions include compositions in which a pharmaceutical, nutriceutical, cosmeceutical, vitamin, foodstuff, antigen, catalyst, cell, nanoparticle, oligonucleotide, gene, extract, cosmetic or fiber is solubilized, protected or dispersed in a solution, particle, emulsion, microemulsion, nanoemulsion, liposome, niosome, molecular matrix or coating including the tocopherol succinic acid derivatives, optionally with other oils, co-solvents, surfactants and cosurfactants. Also described are specific examples of these tocopherol succinic acid derivatives as excipients, surfactants, and as novel therapeutics with biogenic amines, in creams, lotions, soaps, cosmetics, sunscreens, eyedrops, foodstuffs, toothpaste, detergents, emulsions, microemulsions, liposomes, capsules, nanosuspensions, nutriceuticals and injectables. The compositions may be administered orally, topically, or by other nonparenteral routes as can be anticipated by a skilled formulator.

In one embodiment, the composition is a liposome comprising a tocopherol or derivative selected for its chemical stability and its ability to form bilayers alone and in mixtures with phospholipids and cholesterol or phytosterols. Tocopherol derivatives of the present invention form liposomes with novel surface properties.

Importantly, by modifying the surface property of the emulsion droplet or liposome, a therapeutic agent can be directed to the desired cell. The derivatives of the invention can be used to modify the surface properties of emulsion droplets or liposomes so as to produce "stealth" or targeted medicament delivery formulations. Thus, the invention provides tocopherol succinic acid derivatives that are effective in targeted delivery. In one embodiment, the hydrophilic substituent of the tocopherol surfactant is

selected from a list of compounds, such as folate, that are preferentially bound, adhere to, or are taken up by particular types of cells. In this way, the contents of the drug delivery vehicle can be targeted to these cells. Reddy, J.A. and P.S. Low, "Folate-Mediated Targeting of Therapeutic and Imaging Agents to Cancers," *Crit Rev Therapeutic Drug Carrier Systems 15*(6):587-627, 1998.

The present invention also provides compositions containing tocopherol derivatives to deliver any of the biogenic amines, or their amino acid, or peptide precursors. By delivering the compound in the form of an emulsion, the release time of the active compound in the blood can be extended and, by reducing the peak concentration (C_{max}) for the free amine, prevent or modulate systemic or non-specific toxicosis or adverse events.

The tocopherol derivative-containing compositions of the invention can be in the form of an emulsion, microemulsion, micellar solution, liquid crystalline system, self-emulsifying drug delivery system, or a liposomal formulation for parenteral administration, taken here to include intravenous, pulmonary, intraocular, intrathecal, transmucosal, intratracheal, transdermal, subcutaneous, intraperitoneal or intramuscular administration. Oil-in-water, water-in-oil, and bicontinuous (Shinoda, K., et al. "Principle of Attaining Very Large Solubilization," *J. Phys Chem 88*:5126-512, 1984) emulsions, nanoemulsions, microemulsions, as well as liposomes, soaps, and detergents are forms of the invention.

The following non-limiting examples are illustrative of the invention. It will be appreciated that the chemical syntheses described herein can be applied to other tocopherol and benzopyranol succinates.

EXAMPLES

Example 1

Tocopherol Succinate Aspartate

In this example, the synthesis of a representative tocopherol succinic acid derivative, tocopherol succinate aspartate, is described.

Mixed anhydride chemistry was used to synthesize a tocopherol succinate aspartate derivative. D-α-tocopherol succinate was first activated by adding 1.2 equivalents of both isobutylchloroformate (IBCF) and N-methylmorpholine (NMM) in tetrahydrofuran (THF) at -5°C. To ensure complete conversion to the mixed anhydride the reaction was stirred at -5°C for 40 minutes. The mixed anhydride was then filtered to

remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The resulting filtrate was added dropwise to a -5°C solution of 1.0 equivalents of L-aspartic acid dibenzyl ester p-toluenesulfonate salt and 1.3 equivalents of triethylamine (TEA) in THF solution over 1 hour. The reaction was allowed to continue for an additional 1 hour at -5°C; then warmed to room temperature and stirred for 15 hours before isolating the product.

Once the reaction was complete the THF was removed *in vacuo* to yield a crude yellow solid. The product was dissolved in dichloromethane (DCM) and washed twice with saturated $NaHCO_3$, twice with 0.1 N HCl, and once with saturated brine. The resulting organic mixture was dried over $MgSO_4$ and the solvent removed under vacuum to yield an off-white solid, yield 94%.

The tocopherol succinate-aspartate dibenzyl ester was deprotected by hydrogenation to yield the free diacid as a white solid, total yield = 78%. Purity = 95%+ by HPLC analysis. FTIR: amide, ester, amide, and ether (N-H, C=O, C=O, and C-O stretch) are 3336, 1736, 1645, 1153 cm^{-1}, respectively.

The structure was confirmed by LC mass spectroscopy and is shown in FIGURE 3.

Tocopherol succinyl mono- and polyglutamates, or other polypeptide derivatives, can be made by this chemistry. Such compounds have utility as surfactants (detergents), solvents, and bioavailability enhancers for use in soaps, pharmaceutical emulsions, microemulsions, nanoemulsions, SEDDS, cosmetic lotions, and the like.

Example 2

Tocopherol Succinate Tetraglutamate

In this example, the synthesis of a representative tocopherol succinic acid derivative, tocopherol succinate tetraglutamate, is described.

Tocopherol succinate tetraglutamate was synthesized using the methods described in Example 1. However, the linear, unbranched tetraglutamate with free amino terminus (protected as the γ-O-benzyl) was first formed by successive additions of protected glutamate [H-GLU-(γ-O-benzyl)-OH], starting with the monomer. FMOC-GLU-(γ-O-benzyl)-OH was first activated by adding 1.2 equivalents of both isobutylchloroformate (IBCF) and N-methylmorpholine (NMM) in tetrahydrofuran (THF) at -5°C. To ensure complete conversion to the mixed anhydride the reaction was stirred at -5°C for 40 minutes. The mixed anhydride was then filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The resulting filtrate was added

dropwise to a -5°C solution of 1.0 equivalents of H-GLU-(γ-O-benzyl)-OH and 1.3 equivalents of triethylamine (TEA) in THF solution over 1 hour at -5°C. The reaction was allowed to continue for an additional 2 hours, then warmed slowly to room temperature overnight with stirring to complete the reaction before isolating the product. The product was washed and dried and the procedure was then repeated to add a third monomer of H-GLU-(γ-O-benzyl)-OH, and then a fourth. A tetramer of polyglutamate was obtained with 89% purity by HPLC and a yield of about 20%. These reactions may be performed using solid state chemistry instead of FMOC to improve the yield. Some of the product was deprotected by hydrogenation to confirm the identity as shown in FIGURE 4 and having a molecular weight of 534.34.

The remaining tetramer was deprotected at the amine by first dissolving it in DMF, then adding DEA with stirring overnight, followed by removal of the solvent under vacuum. The oil and solid was triturated with 0.1N HCl and washed with diethylether. The remaining solid was then dissolved in DCM and added to the acidic extract. The pH of the aqueous phase was brought to about 13-14 with NaOH and the DCM was separated off. Two more washes with DCM were collected. The organic washes were pooled, washed with saturated bicarbonate and brine, dehydrated over MgSO4, and dried under vacuum to obtain the product with a free terminal amine. The amine is illustrated in FIGURE 5.

Tocopherol succinate mixed anhydride, prepared as described in Example 1, and was added to 1.1 eq. of the tetraglutamate and 1.3 eq. of TEA in anhydrous THF media at reduced temperature. The resulting product was then deprotected by hydrogenation to yield the tocopherol succinate tetraglutamate as the free acid. The structure of tocopherol succinate tetraglutamate is illustrated in FIGURE 6.

Example 3

Tocopherol Succinate Triaspartate: Dendrimeric Tocopherol

In this example, the synthesis of a representative tocopherol succinic acid derivative, tocopherol succinate triaspartate, is described.

Mixed anhydride chemistry was used to synthesize a dendrimeric tocopherol : tocopherol succinate triaspartate. The mono-aspartyl conjugate of Example 1 was first deprotected and then activated by adding 2.4 equivalents of both isobutylchloroformate (IBCF) and 3.0 equivalents of N-methylmorpholine (NMM) in tetrahydrofuran (THF) at -5°C. To ensure complete conversion to the mixed anhydride the reaction was stirred at

-5°C for 40 minutes. The mixed anhydride was then filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The resulting filtrate was added dropwise over 1 hour to a -5°C solution containing 2.2 equivalents of L-aspartic acid dibenzyl ester and 2.5 equivalents of triethylamine (TEA) in THF solution. The reaction was allowed to continue for an additional 2 hours at -5°C, then warmed to room temperature and stirred for 2 hours before isolating the product.

Once the reaction was complete the THF was removed under vacuum to yield a crude yellow oil. The oil was dissolved in dichloromethane (DCM and washed three times with 0.1 N HCl, once with saturated $NaHCO_3$, and once with saturated brine. The dry organic mixture was further dried over MgSO4, then filtered. The solvent removed under vacuum to yield an off-white solid, with significant impurities due to emulsions that formed in the separatory funnel during washing. The product was further purified by column chromatography and about a 1 gm yield at 85% purity was obtained. This corresponded to a yield of about 20% due to losses on the column.

The tocopherol succinate-aspartate dibenzyl ester was then deprotected by hydrogenation to yield the free tetra-acid as a white solid. The structure of tocopherol succinate triaspartate is illustrated in FIGURE 7.

Example 4

Tocopherol Succinate Derivative Having N-CBZ-Ethylenediamine-Linker

In this example, the synthesis of a representative succinic acid derivative, a tocopherol succinate having an ethylenediamine linker, is described.

Synthesis of tocopherol succinate with N-CBZ-ethylene diamine linker was carried out via mixed anhydride chemistry.

The first step involved monoprotection of ethylene diamine (EDA) using benzochloroformate (CBZ). First, 1.7 equivalents of EDA were dissolved in dichloromethane followed by dropwise addition of 1.0 equivalents of benzochloroformate at O°C. The reaction mixture was allowed to stir for 30 mins. at 0°C and 30 mins. at 23°C. This was followed by extraction in. the aqueous layer by addition of 300ml of 0.1N HCl. The aqueous layer was the extracted with 3x100ml dichloromethane (DCM). This was followed by washing with lx saturated NaCl, then drying over $MgSO_4$. The filtrate was dried in high vacuo and refrigerated overnight to afford a crystalline solid of monoprotected ethylene diamine CBZ-$NHCH_2CH_2NH_2$.

The second step involved the covalent coupling of tocopherol succinate with CBZ-$NHCH_2CH_2NH_2$ via mixed anhydride reaction. 1.00 equivalent of tocopherol succinate was activated by adding 1.00 equivalents of isobutyl chloroformate (IBCF) and N-methylmorpholine (NMM) in 100 ml of anhydrous tetrahydrofuran (THF) medium at -5°C. The reaction mixture was stirred at -5°C for 60 mins. The mixed anhydride was filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The filtrate was added dropwise to 1.00 equivalent solution of CbZ-$NHCH_2CH_2NH_2$ in THF containing 1.2 equivalents of triethylamine (TEA) at -5°C. The solution was left stirring overnight. After completion of the reaction, the THF was removed in vacuum and the product was dissolved in DCM and washed with 2x 0.1N HCl, 2X satd. $NaHCO_3$, 2x satd. NaCl. The resulting organic mixture was dried over $MgSO_4$ and dried in high vacuo to yield white solid product. Yield: 97%. FTIR: amine, ester, amide, aromatic, aliphatic (N-H, C=O, C=O, C-H, C-H) are 3317, 1748, 1649, 3066 and 2926 cm^{-1}, respectively.

Example 5

Surfactanticity of a Representative Tocopherol Succinic Acid Derivative

A dendrimeric tocopherol succinic acid derivative including a tri-aspartyl amido group was synthesized and partially purified in Example 3. Surprisingly, the derivative exhibited characteristics of a surfactant, by emulsifying DCM in water, even before the protective groups on the four carboxyl residues were removed. This is a high HLB, low CMC surfactant with a desirable "pizza-slice" shape.

Example 6

Physical Properties of a Representative Tocopherol Succinic Acid Derivative

A tocopherol succinic acid derivative including a mono-aspartyl amido group was synthesized and purified to 97% (w/w) as the sodium salt (TOSA aspartate). The surface tension (γ_s) of a 0.1% solution in water was measured using a K12 Tensiometer (Kruss, Charlotte NC) equipped with a Wilhelmy platinum plate.

Sample	Surface Tension (0.1% w/v, dyne/cm)
Water	71.9
TOSA aspartate	27.4
TPGS	34.5
Amisoft	24.8

For comparison, a non-ionic surfactant TPGS (Eastman, Kingsport TN), and a pure anionic surfactant, Amisoft (Ajinomoto, Tokyo JP), were also tested at 0.1% in water. The aspartate derivative was comparable to Amisoft, an anionic surfactant sold commercially.

Example 7

Tocopherol Succinate Aspartate Emulsion Pre-Concentrate

An emulsion pre-concentrate containing 20 mg/mL amiodarone, a cardiovascular drug, was formulated as follows:

Amiodarone	0.20 gm
Tocotrienols	0.10 gm
Acetylated monoglycerides	0.10 gm
Tocopherol succinate aspartate	0.30 gm
PEG-300	0.50 gm

Purified mixed tocotrienols were obtained from InCon Processing (Batavia IL), and acetylated monoglycerides from Eastman (Kingsport TN). Tocopherol succinate aspartate was synthesized as described in Example 1. Amiodarone and tocopherol succinate aspartate were readily soluble in the tocotrienol oil and did not precipitate when Labrasol and PEG-300 were added. The formulation was warmed to ensure complete dissolution and then cooled to room temperature.

Following this procedure, 0.1 gm of the pre-concentrate was added to 10 mL of buffer ($NaPO_4$ 5 mM, pH 7.4) with gentle stirring. An emulsion with a particle size of

177 nm by PCS was obtained. Emulsion pre-concentrates of this type are useful for oral drug delivery.

Example 8

Tocopherol Succinate Histamine

In this example, the synthesis of a representative tocopherol succinic acid derivative, tocopherol succinate histamine, is described.

Synthesis of the tocopherol succinate histamine derivative was synthesized by activating the d-α-tocopherol succinate with 1.2 eq. of both IBCF and NMM in a THF medium at -5°C for 40 minutes. The (NMM:HCl) was filtered off and the resulting tocopherol succinate mixed anhydride was added dropwise, over a 1 hour period, to a solution of about 4 eq. of histamine (free base) dissolved in 10:1 mixture of tetrahydrofuran/water at -5°C. The mixture .was allowed to react for an additional 2 hours at -5°C, then 1.5 hours at room temperature. The THF was removed under vacuum. DCM was added to extract the crude product, and washed with 2x of saturated $NaHCO_3$ and 1x saturated NaCl solution. The resulting organic layer was dried over anhydrous $MgSO_4$, filtered and removed under vacuum to yield an off-white brittle solid. Yield: 75+%. FT-IR: amide/amine, ester, amide, and ether (N-H, C=O, C=O; and C-0 stretch) are 3208/3077, 1752, 1652, 1150 cm^{-1}, respectively.

Example 9

Tocopherol Succinate Serotonin

In this example, the synthesis of a representative tocopherol succinic acid derivative, tocopherol succinate serotonin, is described.

The mixed anhydride of the d-α-tocopherol succinate was produced with 1.2 eq. of both IBCF and NMM in THF at -5°C for 40-60 minutes. The (NMM:HCl) was filtered off and the resulting tocopherol succinate mixed anhydride was added dropwise to a solution of 1.0 eq. of the serotonin hydrochloride and 1.2 eq. TEA dissolved in 20:1 mixture of tetrahydrofuran/water at -5°C for 1 hour. The mixture was allowed to stir for an additional 1 hour at -5°C and 15 hours at R.T. The THF was removed under vacuum. DCM was added to extract the crude product, and washed with 2x of saturated $NaHCO_3$, 1x 0.1N HCl, and 1x saturated NaCl solution. The resulting organic layer was dried over anhydrous $MgSO_4$, filtered and removed under vacuum to yield an off-white solid, yield 70%. FTIR: alcohol/amide, ester, amide, and ether (O-H/N-H, C=O, C=O, and C-O stretch) are 3393, 1742, 1653, 1154 cm^{-1}, respectively.

Example 10

Bioactivity of a Representative Tocopherol Succinate Derivative

In this example, the bioactivity of a representative tocopherol succinate derivative, tocopherol succinate serotonin, is described.

Serotonin was bonded by an amide bond through the γ-amine to tocopherol succinic acid by mixed anhydride chemistry and formulated for injection in a representative tocopherol formulation (Formulation A) of the invention:

Formulation A

d,1-α-tocopherol	3.0%
Tocopherol succinate serotonin	1.0%
TPGS	3.0%
Poloxamer P-407	1.5%
PEG-400	6.0%

The formulation was processed by microfluidization in a C5 homogenizer (Avestin, Ottawa ON, Canada). An analogous histamine conjugate was similarly formulated (Formulation B), as was a placebo (Formulation C). Three groups of mice received the formulations on Day 1 and Day 2 by tail vein injection. The mice were sacrificed the following day by asphyxiation with CO_2 gas and cardiac blood was preserved by heparinization for white count and differential. Surprisingly, a highly significant difference in white count and differential was noted:

Treatment	Ave. White Count (mm^{-3})
A	12,043
B	9,025
C	9,074

Spleen weights were not significantly elevated in the treatment groups and white cell differentials in the serotonin group showed a dramatic increase in the number of circulating polymoiphonuclear leukocytes. Further work indicated that the serotonin group displayed a rapid and unique response to ischemia, apparently associated with recruitment of adherent neutrophils from the vascular endothelium. In a second aspect of these experiments, sleep latency was also assayed. While most serotonin receptors are believed to require the γ-amine for binding, we felt that peptide-linked conjugation at this amine to a membrane-inserting lipid could result in novel biological properties, either by receptor activation or blockage. Mice received the serotonin compound gd3x. On each day, a striking decrease in sleep latency after startle (reduction in alertness, grooming, and movement) was measured:

Treatment (Day 3)	Sleep latency index [after startle, 0=100% eyes closed in nest]
A	7.5 + 6.5
C	15.0 + 5.8

No cumulative or acute toxicity was observed with this formulation at more than 10 mL/kg, whereas serotonin is highly toxic when given in free, solubilized form.

Example 11

Tocopherol Succinate t-boc-PEG(3400)

In this example, the synthesis of a representative tocopherol succinic acid derivative, tocopherol succinate t-boc-PEG(3400), is described.

Synthesis of t-boc-PEG (3400)-tocopherol succinate was carried out via mixed anhydride chemistry. 0.9 equivalent of tocopherol succinic acid was activated by adding 0.9 equivalents of isobutyl chloroformate (IBCF) followed by addition of 0.9 equivalents of N-methylmorpholine (NMM) in 100 ml of anhydrous tetrahydrofuran (THF) medium at -5°C. The reaction mixture was stirred at -5°C for 60 mins. The mixed anhydride was filtered to remove the N-methylmorpholine hydrochloride salt (NMM:HCl). The filtrate was added dropwise to 1.00 equivalent solution of t-BOC-NH-PEG-NH_2 (Shearwater Polymers Inc, Los Angeles CA) in THF containing 1.0 equivalents of triethylamine

(TEA) at -5°C. The solution was left stirring overnight. After completion of the reaction, the THF was removed in vacuum and a transparent waxy product was obtained which was dried under high vacuo overnight. The t-boc-PEG –tocopherol succinate conjugate was washed with 3x50 ml diethyl ether until a fine free flowing powder was obtained. Yield=79%. FTIR (C=O, N-H, C-H) 1715, 1680,2920 and 2866 cm^{-1}, respectively.

Example 12.

Oral Bioavailability

The tocopherol succinic acid derivatives of the invention can be evaluated as bioavailability enhancers in an in vitro CACO-2 tissue culture model.

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A compound, comprising tocopherol succinate acid covalently coupled to a hydrophilic moiety through a linker moiety, wherein the tocopherol succinate acid is coupled to the linker moiety through at least one of an ester bond or an amide bond, and wherein the hydrophilic moiety associates with water to form a plurality of hydrogen bonds.

2. The compound of Claim 1, wherein the linker comprises an amino acid.

3. The compound of Claim 1, wherein the linker comprises aspartic acid.

4. The compound of Claim 1, wherein the linker comprises a diamine.

5. The compound of Claim 1, wherein the linker comprises ethylenediamine.

6. The compound of Claim 1, wherein the hydrophilic moiety comprises an amino acid.

7. The compound of Claim 6, wherein the amino acid comprises aspartic acid.

8. The compound of Claim 6, wherein the amino acid comprises glutamic acid.

9. The compound of Claim 1, wherein the hydrophilic moiety is no more than weakly bonded to a C8 column packing.

10. Tocopherol succinate tetraglutamate.

11. Tocopherol succinate triaspartate.

12. Tocopherol succinate histamine.

13. Tocopherol succinate serotonin.

14. Tocopherol succinate ethylenediamine.

15. Tocopherol succinate N-protected ethylenediamine.

16. Tocopherol succinate hydroxy-protected polyethylene glycol.

17. An emulsion, comprising a tocopherol succinate amino acid derivative.

18. The emulsion of Claim 16, wherein the tocopherol succinate amino acid derivative is tocopherol succinate aspartate.

19. An emulsion, comprising the compound of any one of Claims 1 to 10.

20. A composition, comprising the compound of any one of Claims 1-16.

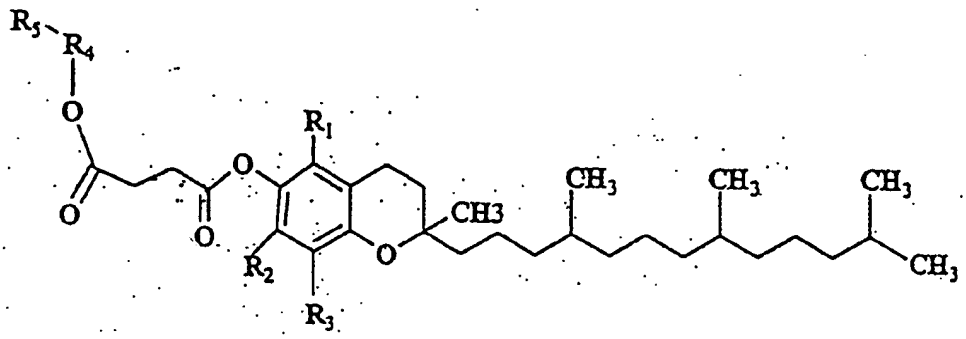

R5
R4
O
O
O
O
R1
R2
R3
CH3
O
CH3
CH3
CH3
CH3

FIG. 1

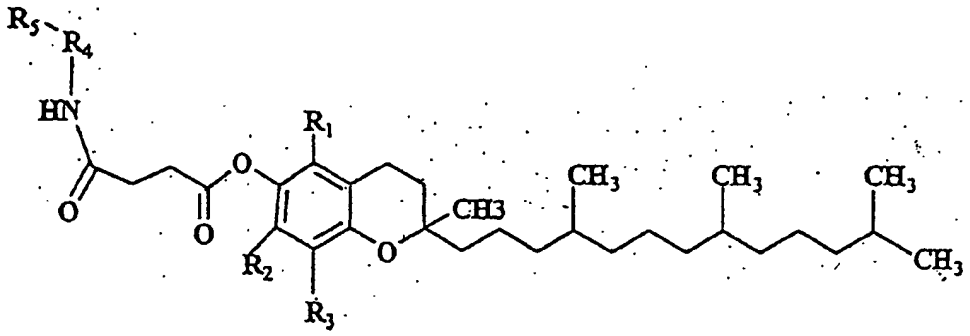

R5
R4
HN
O
O
O
R1
R2
R3
CH3
O
CH3
CH3
CH3
CH3

FIG. 2

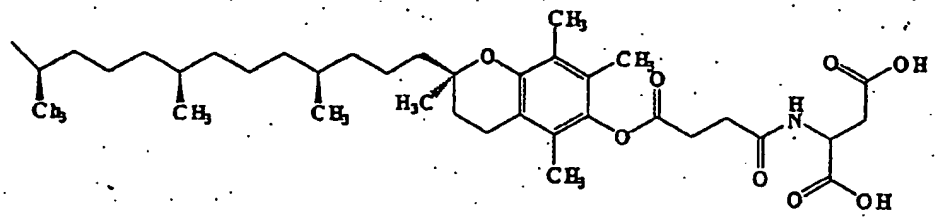


FIG. 3

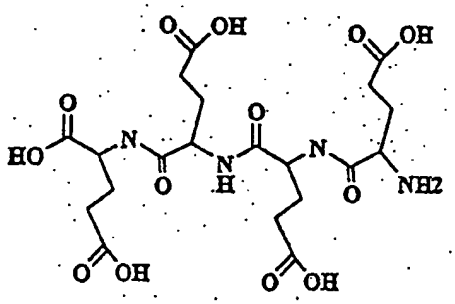


FIG. 4

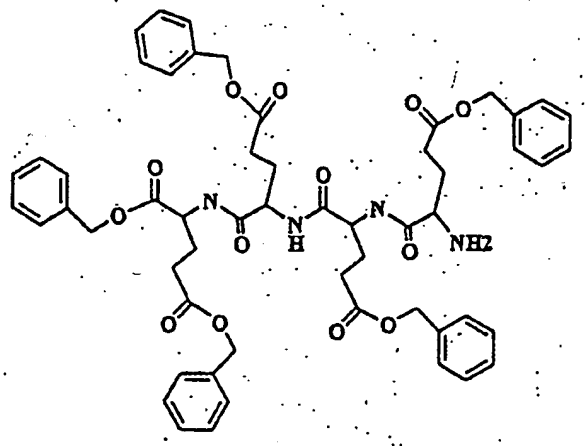

O
O
O
O
O
O
O
O
O
O
O
O
O
O
O
O
N
N
N
H
NH2

FIG. 5

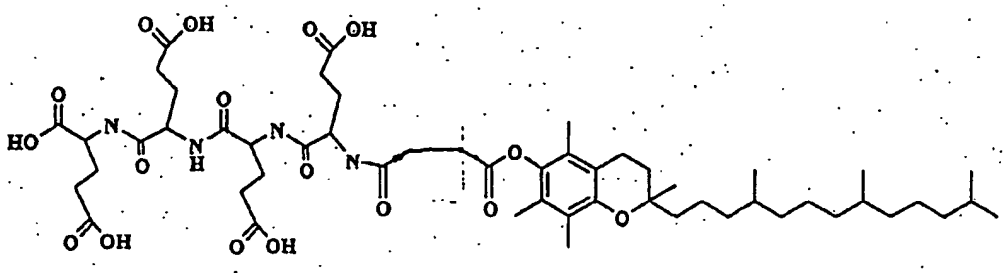

HO
OH
OH
OH
OH
O
O
O
O
O
O
O
O
O
O
O
O
N
N
N
N
H

FIG. 6